REMARKS

This Supplemental Response is being filed to augment the arguments provided to the Examiner in Section V of Applicants' response filed May 18, 2009. Applicants respectfully request that the Examiner consider the remarks below in substitution of Section V found at pages 33-40 of Applicants' Amendment and Response filed May 18, 2009. Applicants have augmented their remarks previously provided to incorporate information provided by Dr. Jozef Arnout in his declaration filed herewith. This Supplemental Response does not replace the discussion relating to unexpected results beginning on page 40 of Applicants' May 18, 2009, response and concluding on page 42, or any other portion of the May 18, 2009, Amendment and Response.

V. Applicants invention is not obvious because it meets a long-felt need in the art for an improved assay for detecting the binding activity between vWF and GP1b, i.e., ristocetin cofactor activity.

Applicants submit that Applicants' invention is not obvious because Applicants' invention satisfies a long felt need in the art for an improved ristocetin cofactor activity assay for diagnosing vWD.

Applicants submit that the requirements for establishing non-obviousness based on long-felt need (See MPEP § 716.04 I) are met. In particular, Applicants submit that the long-felt need for an improved ristocetin cofactor activity assay i) has been persistent and recognized by those of ordinary skill in the art, ii) was not satisfied by others before Applicants' invention, and iii) is satisfied by Applicants' claimed invention for the reasons set forth below.

1. The long-felt need has been recognized by others skilled in the art and has persisted since those skilled in the art recognized the need.

Applicants invented the first assay capable of detecting vWF ristocetin cofactor activity using GP1b(α) presented by an anti-GP1b(α) antibody. Prior to Applicants' invention, detecting the ristocetin cofactor activity of vWF in a sample required using GP1b associated with a platelet. Since the ristocetin cofactor activity assay was first developed in the early 1970s, those

skilled in the art of diagnosing vWD have recognized several deficiencies in the accuracy, sensitivity, and reproducibility of the prior art assays (see Bruguera I, paras. 6-7).

In particular, as stated by Dr. Bruguera in his first declaration ("Bruguera I"):

> "[t]here has been a long-felt need in the field of von Willebrand testing and diagnosis since the time the ristocetin cofactor activity assay was first developed for an improved ristocetin cofactor activity assay that significantly reduces or eliminates intra and interassay variability and improves sensitivity for measuring von Willebrand factor, *i.e.*, an assay that can measure the low levels of von Willebrand factor characteristic of severe von Willebrand disease subtypes with levels of von Willebrand factor below 20%" (Bruguera I, para. 8).

Even as recently as 2007, Favaloro (Favaloro, (2007), *Seminars in Thrombosis and Hemostasis*, 33(8):727-744, "Favaloro II," attached as "Exhibit B" to Bruguera I and Bruguera II) acknowledged that "over the subsequent 35 years or so [since the assay was first introduced], several significant limitations to the ristocetin cofactor assay have emerged" (Favaloro II, pg. 729, RH col.). In particular, as outlined in Favaloro II and described by Dr. Bruguera in his first declaration filed August 20, 2008, ("Bruguera I") at paragraphs 6-7, in his second declaration at paragraphs 5-8 (Second Declaration of Dr. Pablo Bruguera filed herewith, "Bruguera II"), and at paragraph 7 of the Declaration of Dr. Jozef Arnout ("Arnout") filed herewith, the prior art ristocetin cofactor activity assay has suffered from poor interassay variability, poor intra-assay variability, poor reproducibility, and low sensitivity. Poor intra-assay and interassay variability refers to high coefficients of variation (%CV), while low sensitivity indicates that these prior art assays are not sensitive enough to accurately and reliably detect low levels of vWF activity (%vWF) (Bruguera II, paras. 5 and 6; Arnout, para. 10). As a result, prior art ristocetin cofactor activity assays "cannot reliably provide an estimate of von Willebrand factor below around 20%...[which] is a serious limitation" (Favaloro II, pg. 730, LH col.). Given that many vWD patients have vWF activities lower than 20%, 10%, 5%, and even 1%, the inability of the prior art ristocetin cofactor activity assay to reliably detect extremely low levels of vWF activity makes it extremely difficult to accurately diagnose these patients (Bruguera II, para. 6; Arnout, para. 9). This is confirmed by Favaloro who teaches that the limitations of the prior art ristocetin cofactor activity assay described above create a "high potential error rate in terms of false positive and false negative identification of vWD" (Favaloro II, p. 730, RH col., first para.).

The known limitations of the prior art ristocetin cofactor activity assay in diagnosing vWD have been acknowledged by others in the art, not just Favaloro and Dr. Bruguera. For example, the National Institutes of Health December 2007 Report entitled "The Diagnosis, Evaluation, and Management of von Willebrand Disease" ("NIH Report," attached as "Exhibit B") states that:

> "The ristocetin cofactor activity (VWF:RCo) assay has high intra and interlaboratory variation…the coefficient of variation (CV) has been measured in laboratory surveys at 30 percent or greater…This becomes important not only for the initial diagnosis of VWD, but also for determining whether the patient has type 1 versus type 2 vWD" (NIH Report, page 27, LH col.).

Further, the NIH Report confirms Favaloro's assertion that the prior art ristocetin cofactor activity assays lack sensitivity to low levels of vWF and cannot accurately detect below 10-20% vWF. In particular, the NIH report asserts that "the CV is still higher [than 30%] when the vWF is lower than 12-15 IU/dL [(12-15% vWF)]" (NIH Report, page 27, LH col.). In other words, for vWF below 15%, the coefficient of variation is greater than 30%. Having a coefficient of variation greater than 30% indicates that these assays are not precise enough to give accurate measurements at these lower levels of vWF, as the generally accepted industry standard for variation in these types of assays less than 15% (Bruguera II, para. 9). Such an error level would significantly and negatively effect the accuracy of a diagnosis of vWD (Bruguera II, para. 9).

Further, Kitchen *et al.* ((2006), Sem. Thromb. Haemost., 32:492-498, attached as Exhibit C to Declaration of Dr. Arnout, filed herewith), like Favaloro, and the NIH, reports that the coefficients of variation for the prior art ristocetin cofactor assays are too high. For example, Kitchen gathered data from 128 laboratories testing the ristocetin cofactor activity of the same 10 samples of vWD patient plasma over the period from 2001-2005 (abstract; pg. 494, LH col., first para.). Kitchen reports for the vWF ristocetin cofactor activity assay that:

> "In the past, there has been poor agreement between results in different centers using the same technique, as indicated by high CVs. We have previously reported CVs up to 64% even among an expert group of International Haemophilia Training Centers…There has been no improvement during 2001-2005, when average CVs of 40% to 50% were noted for uses of

aggregometry and visual agglutination" (Kitchen, pg. 497, RH col., 1st para.).

Kitchen's data presented in Table 2 (pg. 494) indicate that various methods of detecting vWF ristocetin cofactor activity had coefficients of variation ranging from 12.0-69.2% (average 51.5%), 22.5-104% (average 49.1%), and 15.7-98.5% (average 41.3%), depending on the method used to detect vWF activity. As indicated by Dr. Arnout, the extremely high levels of inter-laboratory variation reported for the assays assessed by Kitchen indicate that these assays cannot provide accurate and reliable measurements of vWF, especially at low levels of vWF, *i.e.*, below 10%-20% vWF (Arnout, para. 6).

Further, based on Kitchen's data, he concludes that "[his] surveys indicate that laboratory testing of vWF remains problematic. Results are imprecise by most of the techniques currently used. It remains to be seen whether some newer techniques will offer consistently improved precision" (Kitchen, pg. 498. LH col., last para.). Accordingly, Applicants' submit that these conclusions of Kitchen, including Kitchen's data, and the conclusion of Dr. Arnout regarding Kitchen's data further evidence the need for an improved ristocetin cofactor assay having the sensitivity to detect low levels of vWF with the appropriate low levels of variation.

Applicants submit that the need for an improved ristocetin cofactor activity assay has persisted since the deficiencies with the assay were first recognized in the early 1970s (Bruguera II, para. 7; Arnout, para. 11). For example, that Favaloro, the NIH Report, and Kitchen were complaining about the deficiencies of the prior art ristocetin cofactor activity assay as recently as 2006 and 2007 indicates that the assay's problems still persist. In addition, as stated by Dr. Arnout and Dr. Bruguera, "Since the 1970s when the prior art vWF:RCo (vWF ristocetin cofactor activity) assay was first developed, there has been a persistent need for a vWF:RCo assay that addresses the problems with the prior art VWF:RCo assay" (Arnout, para. 11, Bruguera, para. 7). Further, according to Drs. Arnout and Bruguera, until the advent of Applicants' claimed invention, the known problems with the prior art ristocetin cofactor assay had not been satisfactorily addressed (Arnout, para. 12; Bruguera II, para. 10).

For all these reasons, Applicants submit that since the 1970s, there has been a recognized and persistent need in the art by skilled artisans for an improved ristocetin co-factor activity

assay having the sensitivity to detect low levels of vWF and with reduced levels of intra and interassay variability.

*2. **Others had not satisfied this long felt-need prior to the invention by Applicants.***

Applicants submit that others had not satisfied the need for an improved ristocetin cofactor activity assay as of the priority date of this application.

Others have made attempts to improve the prior art ristocetin cofactor assay in the years since the deficiencies of the assay were first elucidated (Arnout, para. 12; Bruguera II, para. 10). For example, laboratories have used stabilized platelets obtained from suppliers rather than preparing their own platelets in order to reduce the variability caused by each laboratory using platelets from different sources (Arnout, para. 12; Bruguera II, para. 10). Further, laboratories have automated the prior art ristocetin cofactor assay to reduce error in detecting agglutination of platelets, to reduce the time-intense nature of the assay, and to computerize the complex series of calculations involved in measuring platelet agglutination to reduce human error (Arnout, para. 12; Bruguera II, para. 10). However, as stated by Drs. Arnout and Bruguera, none of these efforts to improve the prior art ristocetin cofactor activity assay has been successful in providing the needed improvements in inter- and intra-assay variability and sensitivity (Arnout, para. 12; Bruguera II, para. 10).

For example, an exemplary commercially available ristocetin cofactor activity assay, available as early as January 1999 (Bruguera I, paras. 9-16 and "Exhibit C"), and discussed by Dr. Bruguera in his first declaration had not solved the problems of the prior art ristocetin cofactor activity with respect to inter-assay and intra-assay variability and sensitivity (see also Arnout, para. 14-15). While the exemplary commercially available assay may have improved inter-assay and intra-assay variability due to performance of the assay in an automated system, "even automated assays…have not fully addressed all the drawbacks of the classical ristocetin cofactor assay" (Arnout, para. 13; Bruguera I, para. 10). For example, as stated by Favaloro, "automation of test procedures (using instrumentation) has certainly reduced the ristocetin cofactor activity assay's intra-assay and inter-assay variability, ***but has not alleviated the issue of low-level assay sensitivity***, nor does automation seem to protect laboratories against vWD

identification errors" (Favaloro II, pg. 730, LH col., emphasis added). Each of Dr. Arnout and Dr. Bruguera agree with Favaloro that automation alone has not corrected the deficiencies of the prior art ristocetin cofactor activity assay and that further improvements were necessary as of the priority date of Applicants' claimed invention (Bruguera I, para. 10, Arnout, paras. 13 and 15).

As indicated by Favaloro, an assay with the ability to accurately detect vWF activity below 20% is needed (Favaloro II at 730, LH col.). The product specifications for the commercially available assay discussed by Dr. Bruguera provides no indication that it can accurately detect levels less than 20% vWF; in fact, the product specification does not indicate the lower limit of quantitation for the assay (Arnout, para. 14; Bruguera II, para. 12). In addition, the coefficients of variation for the commercially available assay's pathological control, *i.e.*, a sample with a known low level of vWF, ranges as high as 16.2% (within run) 16.9% (total) (Arnout, para. 14; Bruguera II at para. 12). According to Drs. Arnout and Bruguera, it would not be possible to accurately and precisely quantitate low levels of vWF, *i.e.*, below 10-20% vWF, because the coefficients of variation for this assay exceed the generally accepted industry standard for precision which is less than 15% CV (Arnout, para. 15; Bruguera II at para. 12). Accordingly, "further improvements in inter-assay and intra-assay variability were still necessary as of the priority date" of this application (Arnout, para. 15; Bruguera I, para. 10).

In addition, despite the known deficiencies in the prior art ristocetin cofactor activity assay's ability to provide reliable detection of vWF activity, the NIH Report indicated in 2008 that the prior art ristocetin cofactor assay is still "the most widely accepted laboratory measure of vWF function" (NIH report, page 27, sentence bridging LH and RH col.). That these assays are still being used despite their deficiencies is further evidence that others have not satisfied the need for an improved assay, as there is no satisfactory alternative. Moreover, that Kitchen, Favaloro, and the NIH Report have both reported the deficiencies of the prior art ristocetin cofactor activity assays as recently as 2006 and 2007 is further evidence that the prior art ristocetin cofactor assay has not yet been satisfactorily improved by others. In fact, Kitchen specifically acknowledged in 2006 that the "laboratory testing for vWF remains problematic," that "[r]esults are imprecise," and that "it remains to be seen whether some newer techniques will offer consistently improved precision" (Kitchen, pg. 498, LH col., last para.), indicating that

need for an improved ristocetin cofactor activity assay has not been met. Furthermore, as stated by Dr. Arnout, "the prior art vWF:RCo assays available prior to the earliest effective filing date of Applicants' claimed invention and even available today have not satisfactorily resolved the serious problems inherent in the prior art VWF:RCo assays."

For all these reasons, Applicants submit that others have not yet satisfied the long felt need for a ristocetin cofactor activity assay with the necessary improved sensitivity to accurately detect low levels of vWF and to eliminate the very high coefficients of variation typically seen with the prior art ristocetin cofactor assays.

3. Applicants' invention satisfies the long felt need of others skilled in the art.

While Applicants' claimed assay is not yet available commercially, those of skill in the art who are aware of Applicants' assay acknowledge that it fulfills the long felt need of skilled artisans in the field of vWD diagnosis for a more precise and accurate assay for detecting ristocetin cofactor activity. For example, Drs. Arnout and Bruguera have indicated that until the advent of Applicants' claimed invention, the known problems with the prior art ristocetin cofactor assay had not been satisfactorily addressed (Arnout, para. 12; Bruguera II, para. 10).

As indicated by Drs. Arnout and Bruguera, Applicants assay has satisfied this long felt need by providing further reductions in the coefficients of variation as compared to prior art ristocetin cofactor activity assays, and by providing the requisite low level sensitivity to detect levels of vWF below 20%, 10% and 1% and even 0.5% vWF (see Bruguera II, para. 16; see Arnout, para. 17).

For example, in contrast to the prior art ristocetin cofactor assays which "cannot reliably provide an estimate of vWF below around 20%" (Favaloro II, p.730, LH col., 1st para.), Applicants' claimed assay can provide detection levels at least as low as 1% vWF, overcoming the "serious limitation" of the prior art assay acknowledged by Favaloro (Favaloro II, pg. 730, LH col., 1st para.). In fact, Applicants' assay has a lower limit of quantitation of 0.27% vWF with a coefficient of variation of 7.9%, well below the generally accepted industry stand of 15% CV (Bruguera II, paras. 8 and 11, Arnout, para. 17). Further, Applicants' lower limit is 30-60 times lower than the 10-20% lower limit of the prior art ristocetin cofactor activity assay as reported by Favaloro (Favaloro II, p.730, LH col., 1st para.). As stated by Drs. Arnout and

Bruguera, this is the lowest known lower detection limit for any ristocetin cofactor activity assay available as of the priority date of this application (Arnout, para. 17; Bruguera II, para. 11). Accordingly, as indicated by Drs. Arnout and Bruguera, Applicants' assay meets the long felt need in the art for a ristocetin cofactor activity assay that can detect less than 10-20% vWF and lower than 1% v WF as Applicants assay is 30-60 times more sensitive than the lower detection limit of 10-20% for prior art ristocetin cofactor assays as reported by Favaloro (Favaloro II, pg 730, LH col. 1st para.) (see Arnout, paras. 11, 17, and 19; Bruguera II, paras. 7, 11, and 16).

Further, Applicants assay provides reductions in coefficients of variation beyond those of the prior art ristocetin cofactor activity assays. Coefficients of variation for Applicants' assay are shown in Table 1 of Dr. Bruguera's second declaration (Bruguera II, Table 1). As stated by Favaloro, coefficients of variation for prior art ristocetin cofactor activity assays are reported in the range of 20-40% (Favaloro II, LH col., 3rd para.). As stated by Drs. Arnout and Bruguera, these lower coefficient of variation obtained from Applicants' claimed assay, in comparison to the coefficient of variation for the prior art ristocetin cofactor activity assays, including the exemplary commercially available ristocetin cofactor activity assay discussed above, demonstrate a significant and valuable improvement in reducing the inter- and intra-assay variability of the ristocetin cofactor activity assay (Arnout, paras. 15-16; Bruguera, para. 14). Applicants improvements in reducing total and within run variation indicates that the mean % vWF activity detected in patient samples according to Applicants' claimed method with be more accurate, and as a result, a more accurate diagnosis of vWD can be made (Arnout, para. 16; Bruguera II, para. 14). Accordingly, for these reasons which are supported by the declarations of Drs. Bruguera and Arnout, Applicants have met the long-felt need in the art for a ristocetin cofactor activity with reduced levels of variability (see Bruguera II, para. 16; Arnout, para. 19).

Further evidence that Applicants' assay has met the long-felt need is found in the NIH Report which refers to ELISA assays that assess direct binding of a person's plasma vWF to GP1b derived from plasma glycocalicin (pg. 27, LH col.). In support of this assay, the NIH Report references a paper published in 2000, after the priority date of this application, which is authored by the inventors of this application and which generally describes the claimed invention (Deckmyn *et al.*, (2000), "A reliable and reproducible ELISA method to measure ristocetin

cofactor activity of von Willebrand Factor," Thromb. Haemost., 83:107-113, attached as Exhibit C). The NIH Report acknowledges that Deckmyn's method, which is the subject of the instant application, can detect below 1U/dL of vWF (*i.e.*, below 1% vWF), see Deckmyn 2000 at 112, LH col.). The other assays reported by the NIH, which require use of platelet-GP1b, have sensitivity limits of 6-12 IU/dL and 10-20 IU/dL, but with coefficients of variation greater than 30% in these ranges, especially when measuring below 12-15 IU/dL (NIH report, pg. 27, LH col.). As previously discussed, these high coefficients of variation mean that these prior art assays lack the necessary sensitivity to precisely and accurately detect below 10-20% vWF. This contrasts with Applicants' assay, which has coefficients of variation ranging from 3.5%-7.2% (Bruguera II, Table 1). That the NIH report has acknowledged the increased sensitivity of Applicants' claimed invention over the other assays is evidence that Applicants' assays has met the long-felt need in the art.

Applicants submit that as of the priority date of this Application, Applicants' claimed invention was the only known ristocetin cofactor activity assay with the sensitivity to accurately detect less than 1% vWF with acceptable levels of inter-assay and intra-assay variation (CV%) (Arnout, para. 19; Bruguera II, para. 16). Further, because Applicants' claimed assay has the ability to reliably detect levels of vWF well below 1%, *i.e.*, as low as 0.27%, and because it significantly reduces levels of intra-assay and inter-assay variability, the long felt need in the art for an improved ristocetin cofactor activity assay with the requisite sensitivity to precisely and accurately detect low levels of vWF with satisfactorily low coefficients of variation has been met by Applicants' claimed invention, a conclusion supported by the declarations of Drs. Bruguera and Arnout (see Bruguera II, para. 16; Arnout, para. 19).

Further, Applicants submit that the improvements in inter-assay and intra-assay variability as well as sensitivity achieved by Applicants' assay result from the limitations of the claimed invention. As stated by Dr. Bruguera, the success of Applicants' assay "can be attributable to the use of a soluble form or portion of the glycoprotein 1b(α) presented by an anti-GP1b(α) antibody" (Arnout, para. 18; Bruguera II, para. 15).

Applicants further submit that the patentability of Applicants' claimed invention is evidenced by the fact that in all the years intervening since the development of the ristocetin

cofactor assay, no one has attempted to improve the ristocetin cofactor assay by using a soluble form or portion of glycoprotein 1b(α) that is not associated with a platelet, let alone an assay where the soluble form or portion of GP1b(α) is presented by an anti-GP1b(α) antibody. If such an improvement were obvious, Applicants respectfully question why no one else had done so prior to Applicants' earliest effective filing date.

4. Conclusion

For all the reasons set forth above in Sections V(1), (2), and (3), Applicants submit that their claimed invention is not obvious because it meets a long felt need in the art for an improved ristocetin cofactor activity assay capable of accurately and precisely detecting extremely low levels of vWF (*i.e.*, less than 10-20%) with acceptable levels of inter-assay and intra-assay variation (CV%), a need not previously met by others, a need that has been recognized since the early 1970s, conclusions which are supported by the declarations under 37 C.F.R. § 1.132 of Drs. Bruguera and Arnout. Accordingly, Applicants respectfully request that the rejection of the pending claims under 35 U.S.C. 103(a) be reconsidered and withdrawn.

CONCLUSION

Applicants believe that the pending claims are now in condition for allowance. The Examiner is invited to telephone the undersigned attorney to discuss any remaining issues. Early and favorable actions are respectfully solicited.

Respectfully submitted,

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